

THE CONDITIONS FOR CONJUGATION IN DIVERSE RACES OF PARAMECIUM

BY

HOYT S. HOPKINS

(From the Zoölogical Laboratory of the Johns Hopkins University)

DISSERTATION

Submitted to the Board of University Studies of the Johns Hopkins University
in conformity with the requirements for the Degree of Doctor of Philosophy.

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CONTENTS

Introduction.....	339
Material and methods.....	344
1. Conjugation in diverse strains.....	347
a. <i>Paramecium caudatum</i>	347
b. <i>Paramecium aurelia</i>	356
2. Growth factors influencing conjugation.....	368
General summary.....	374
Literature cited.....	376

INTRODUCTION

Maupas ('89) was one of the first to suggest differences between nearly related species of Infusoria in respect to their tendency to conjugate. He found, for example, that *Colpidium colpoda* would conjugate more readily than the similar species *C. truncatum*. Like differences have been shown by later investigators to obtain in other genera, as in the two species of *Colpoda* studied by Enriques ('08). It had long been known, too, that cultures of the same species differ much as regards susceptibility. Most authors attributed these differences to environmental factors, as mixed cultures of uncertain derivation were used in most cases. As pure, or 'pedigreed,' races began to be used, these cultural diversities were conceived of as having possibly a racial basis. If diversities with respect to size, shape, and division rate have their origin in heredity, may it not be that differences in the ability to conjugate are based upon hereditary factors?

This view has led to some controversy in recent years in regard to the existence of so-called 'non-conjugating races,' calling into question the universality of fertilization among the Infusoria.

That the process may be postponed indefinitely in *Paramecium aurelia*, provided the cultural conditions are kept uniform and favorable, seems probable in the light of Woodruff's extended work, for he maintained one strain through more than six thousand generations without conjugation (1917). This same race of organisms was subjected to conditions which favor conjugation with positive results (Woodruff, '14). It would appear from these results that, in *Par. aurelia* at least, the occurrence of conjugation is facultative under uniform conditions of environment. Other reports, moreover, would indicate that conjugation is possibly lacking in the life-history of some races of *Paramecium*, or recurrent only after very long time intervals in others. Thus Jennings ('10) maintains that there exist in these organisms striking racial differences with respect to conjugation. In some of the strains employed in his work normal 'epidemics' occurred frequently, sometimes apparently under uniform conditions of existence, in some only infrequently, and in others not at all. He emphasized also growth and nutrition as important factors in regulating conjugation. Thus a period of rapid multiplication followed by a decline in the division rate usually gave an epidemic of conjugation in the majority of cases. A preliminary period of semistarvation (reduction in division rate) of two weeks or longer made the organisms more susceptible when the above-mentioned procedure was followed. Some few cultures (races) failed to yield conjugants even when the most favorable conditions were afforded. Two species of *Paramecium* (*caudatum* and *aurelia*) were used in his work, and the same kind of differences was found in each: there were races which conjugated freely, often at frequent intervals, others which did not, although *P. caudatum* showed less frequent epidemics and was characterized by a greater number of the 'non-conjugating' races.

The process of nuclear reorganization, or endomixis, discovered by Hertwig ('89), and worked out by Woodruff and Erdmann ('14, '16), would seem to suffice in maintaining the normal vegetative processes in *Paramecium* in the entire absence of conjugation. Even in such a form as the hypotrichate *Uropeltus*, according to the work of Calkins ('19, two papers),

conjugation is probably not essential to the continued life of the race. For, although a nuclear reorganization process occurs only within the cyst, animals can be derived from the cysts which develop with the same renewed vigor as characterizes exconjugants.

The direct effect of altered environmental conditions in determining conjugation was more strongly emphasized by Enriques ('09), who employed solutions of electrolytes to induce epidemics of conjugation in *Cryptochilum nigricans*. His studies led him to believe that the process is in no way bound up with an inferred 'life-cycle,' and so is not dependent primarily upon internal factors. This same general method was adopted later by Zweibaum ('12) in a careful study of a race of *Paramecium caudatum*. During the early history of this race conjugation could be induced at times simply by subjecting samples of the flourishing cultures to the action of certain salt solutions. Later it was found necessary to subject the cultures to a preliminary period of partial starvation ('disette') lasting from five to six weeks. Cultures which had been maintained under conditions of steady growth ('cultures continuatives') no longer gave conjugants when treated with salts, except in rare instances, and then in very small numbers. From the 'dormant' cultures, however, conjugants were readily obtained after allowing the organisms to multiply rapidly in a rich culture medium; changed every three or four days during a period of about one week, then subjecting them to the action of the salt solutions in optimal concentrations (as determined previously by experiment). This last process is to be carried out with large numbers of paramecia, in a small culture dish, the organisms being thus limited to a small amount of nutriment. The solutions of salts are then added in the ratio of three parts of salt solution to one part of culture (15 cc.: 5 cc.). The essential steps in the procedure are seen to be those laid down by Maupas—with an added factor, namely, the treatment with a chemical reagent. In general the chlorides of aluminium, gold and ferric iron, and the salts of sodium, were found to be most effective. Just what rôle the electrolytes may serve, whether as an additional stimulant or in some other

more specific way, is not clear. Hypertonic solutions of 'tap-water' or of glucose produce like results to a lesser degree, but they act apparently by increasing the osmotic concentration of the medium, so that their effect is probably not a specific one in the chemical sense. The result produced by any one of these reagents is to augment the intensity of conjugation (increase the proportion of conjugating animals), for a small percentage of individuals may conjugate in a culture to which water alone has been added (in place of salt solution), or sometimes even in the main culture which had been renewed with hay infusion.

In the light of this recent work in which refined methods of experimentation were employed successfully with one race, the question has been asked whether we cannot by such means induce conjugation in any race of infusoria. As applied to *Paramecium* in particular—are there any racial differences in respect to conjugation, or are the alleged diversities environmental in origin? According to Zweibaum, we ought to be able to induce conjugation in any race of *Paramecium caudatum* by using those methods which he finds most effective, for he holds that all supposed racial diversities, such as those described by Jennings and Hargitt ('10) for size, result from cultural differences. To state his position more clearly, I quote from page 276 of his paper:

Dernièrement encore pour les *Paramaecies* Jennings détermine diverses races—injustement à mon avis—et affirme que les conditions de conjugaison diffèrent considérablement selon ces races. L'auteur déplace ainsi le centre de gravité de la question sans ajouter une conception nouvelle et un déterminisme positif dans les considérations de conjugaison chez les *Paramaecies*.

And again, in summarizing the conditions which he considers sufficient for conjugation in *Par. caudatum*, he writes (p. 292):

Comme conclusion nous pouvons dire que les *Paramaecies* que Jennings et Hargitt croyent appartenir à des races diverses, se conjugueront toujours, lorsque après avoir passé par un état de disette prolongée de 5-6 semaines, on remplira les autres conditions nécessaires—à savoir—la disette comme agent de l'action instantanée, la température de 20-23°C. et la composition du milieu (voir 2ème partie).

Ce qu'on dit être des races diverses de *Paramaecium* n'est que le résultat de la quantité de la nourriture.

It is apparent from these statements that Zweibaum holds that the conditions for conjugation as worked out for one race of *Paramecium* apply to all other races of the same species—at least in *P. caudatum*; and he strongly inclines toward the view that conjugation is regulated primarily through environmental conditions. This, at any rate, is the view expressed by Enriques (on the basis of Zweibaum's work¹), for he did not see fit to change his original point of view, believing that the preliminary period of semistarvation of five to six weeks to which the paramecia were subjected was but a step in the experimental procedure, making the organisms more susceptible to the effects of the salts.

The present study was undertaken mainly with this object in view: to ascertain whether the so-called 'conditions for conjugation' worked out for one race of *Paramecium* are effective for other races and, in general, to determine so far as possible the differences in the conditions required to induce conjugation in different races. An attempt was made, also, to find out whether the various races under observation were characterized by different time intervals between conjugation periods, or, as we might say, by diverse 'sexual periodicities.' Jennings ('10) called attention to such apparent periodicities in certain races of *P. aurelia*. His studies on *P. caudatum*, while less extensive, indicated that in some races of this species conjugation occurs at intervals of several months, or perhaps years. The importance of further work relative to this point was felt more keenly in view of the greater perfection attained in the experimental (or environmental) study of conjugation. Even in other groups of organisms the study of natural periodicities in respect to sexual activity, apart from environmental influences, has not received the attention which it merits.

This work was undertaken at Dr. H. S. Jennings' suggestion, with this end in view. I wish here to express my thanks to Professor Jennings for his kindly advice and criticism, as well as for assistance in the procuring and rearing of material for cultures.

¹ See Zweibaum ('12), footnote by Enriques, pp. 346-347.

For our purpose, then, three general questions should be kept in mind: 1) Do there exist differences between various races of *Paramecium* with respect to conjugation? 2) If so, what are they? 3) From a comparative study of different races of the same species and of the different species, what can be said in general regarding the nature of the conditions for conjugation in the Infusoria?

Any answer to the third question must depend, in part at least, upon the facts brought out in connection with the other two. If constant natural differences in the time interval between successive epidemics of conjugation in different races and species can be demonstrated, it would coincide better with the view that conjugation is primarily a function of some internal factors, although, of course, it would not exclude the setting in operation of these factors by environmental conditions. The complete subordination of conjugation to direct environmental conditions might lead us to regard the process as mainly environmental in its origin, and perhaps directly adaptive in its functional significance.

MATERIAL AND METHODS

Twenty-four races of *Paramecium*, each derived originally from a distinct individual, were used in the course of this work; eleven representing *P. caudatum*, and thirteen, *P. aurelia*. Many other races were isolated, but owing to unfavorable culture conditions died out before reaching the 'mature' condition of old cultures.

This dying out of unbalanced cultures is conditioned largely by the racial characteristics of the protozoan isolated, it being ill adapted to the conditions of growth supplied in the laboratory. This is shown by the fact that other races which were reared with difficulty to the condition of stable cultures in the beginning were found to show, some months later, this same inability to develop rapidly when isolated individuals were reared again under rich culture conditions. Deleterious strains of bacteria undoubtedly serve as the immediate cause of the death of a culture in most cases, but the diversities between races of pro-

tozoans as regards resistance must be taken into account in order to understand why one succumbs in an environment in which another grows and apparently thrives. If two races distinct in size, one of *Par. caudatum*, the other of *aurelia*, are reared together in the same culture dish, hence in the same medium, one may survive, the other die, or both may live together. Like differences undoubtedly exist between races of the same species, for my work indicates that such races show constant natural differences as regards their resistance to specific reagents and their ability to grow readily in slide cultures as well as in mass cultures.

During the course of development of these cultures records were taken of races under similar growth conditions, in order to determine in advance which ones were subject to periodic epidemics of conjugation and the nature of these rhythmic changes, so as to compare their behavior under usual conditions with that to be obtained later in the experiments. Certain cultures were subjected to daily observation for weeks at a time with this object in view, and record kept of sporadic occurrences and of conjugation en masse. This revealed what races were subject to frequent epidemics and in what ones conjugation was rare or lacking. Most of these observations were made daily under a binocular microscope of wide field of vision, upon cultures growing in Stender dishes measuring 5 cm., 6 cm., and 10 cm. in diameter, so that any but rare conjugating pairs could hardly be overlooked. The same method of daily observation was adopted during the course of experiments with small cultures, and since these cultures were comparatively free from sediment, it was an easy matter to conduct thorough observations upon them during the few days required for each experiment.

Many of the experiments referred to in this paper were carried out after the procedure recommended by Zweibaum (p. 341), using salt solutions, with the object of showing whether each race would not yield conjugants when the conditions which he found most favorable were supplied. For this purpose subcultures were derived from the main cultures by diluting 50 to 100 cc. of the culture containing many paramecia with an equal

part of distilled water. These were allowed to stand for several weeks as 'dormant cultures,' with very little nutriment, at the end of which time hay infusion was added. Such cultures to which hay infusion has been added to induce rapid multiplication will be referred to as 'renewed cultures.' After about the fifth day portions of these cultures were treated with salt solutions in small Stender dishes.

The renewed cultures of *P. aurelia* often yielded about as many conjugating pairs as the smaller (experimental) cultures derived from them, and treated with salt solutions. It was found further that certain inorganic salts added to the renewed cultures directly, i.e., immediately following the period of dormancy, at the time of renewal, made these organisms (*P. aurelia*) more susceptible to conjugation after the preliminary period of multiplication of about five days. Small watch-glass cultures derived from these usually yielded conjugants earlier—after the third or fourth day following renewal of the main culture—but the renewed cultures themselves often yielded just as many pairs after the fifth day, showing that the sudden change brought about through such 'isolation experiments' is not an essential condition for the appearance of conjugation.

In general it may be said that large cultures of *Paramecium*, of either species, give fewer conjugating pairs than small cultures of the same race when treated in a similar manner. This condition may perhaps be attributed to the greater accumulation of organic matter (excretion products) in large cultures than in small and in part to the more uniform conditions.

The experimental procedure will be described in greater detail in connection with the experiments themselves. The culture medium used throughout during the course of this work on *Paramecium* consisted of hay infusion. 'Tap-water' was used to maintain the stock cultures, which were contained in battery jars and kept at room temperature. Cultures intended for use in future experiments were diluted with distilled water just before being subjected to their period of dormancy, and at the end of this period were renewed with an equal part of hay infusion in distilled water, changed subsequently every three to four days.

So after the multiplication period of five days or longer the organisms to be subjected to the treatment with salts were in essentially a distilled water medium. Spring water from a perpetual spring near the Johns Hopkins University campus was found to be most favorable for maintaining *Paramecium* lines on depression-slides (as slide cultures), for the organisms are very sensitive to sudden changes in the character of the medium such as attend this method.

The various strains of distinct origin are designated each by a separate number, usually followed by a small letter. Cultures of the same strain or race derived each from one individual of the original culture (frequently from an exconjugant), are designated by the same number followed by a different letter. Subcultures are often distinguished by Roman numerals.

1. CONJUGATION IN DIVERSE STRAINS

a. Paramecium caudatum

I shall take up first the question of conjugation in races of *P. caudatum*, because it was in a race of this species that Zweibaum worked out the conditions which he claims to be equally applicable to any race of this species. For, if his conclusion is valid, it should be possible to repeat his experiments upon various other strains under parallel conditions, and thus show definitely that there are no inherent diversities with respect to conjugation. On the other hand, if two races which have had the same environmental history, react differently under these same experimental conditions, one giving conjugants, the other not—or even if they react differently to other conditions which have been shown to favor conjugation in some one race—then we have evidence for racial diversities.

The following series of experiments with three races of *P. caudatum* are illustrative of others which were carried out at other times with the same races under less parallel conditions. Experiments with salt solutions found by Zweibaum to be most effective in his work were conducted upon each strain on the same days (table 1). Cultures *6aII* and *5aII* had been dormant since

March 28, 1919. A culture, *17aII* (containing also individuals of a race of *P. aurelia*, *13a*), had been dormant since April 5th. On May 8th the first of these cultures was renewed, and on the following day the other two cultures. Experiments were set on May 13th from samples of each culture, using NaNO_3 and FeCl_3 solutions; and these gave, in the case of *5aII* and *6aII* no conjugants, in *17aII*, however, conjugation to the extent of 30 to 50 per cent (May 14th). Conjugants were found in smaller numbers (2 to 5 per cent) in the renewed culture of *17aII* to to which no salts had been added, so that the results obtained with this race are clearly comparable to those obtained by Zweibaum in his experiments: the effect of the salts is to augment the intensity of conjugation.

This same race had shown its ability earlier to yield conjugants, when another culture, *17aI*, was renewed after about ten days of semistarvation (conjugation on February 26th). Similar results were obtained with a third culture of this race, *17aIII*, on April 10th and 11th, when renewed after fifteen days of dormancy. Other cultures of *17aIII* (containing also individuals of *P. aurelia*) likewise gave conjugation (table 2).

That the negative results obtained in the above experiments with cultures *5aII* and *6aII* cannot have been accidental (resulting perhaps from unfavorable culture conditions) seems certain from the fact that similar experiments upon other portions of these cultures, renewed after longer or shorter periods of dormancy, all proved negative (table 1). The further history of these two races may also be referred to in support of this view. Both were isolated from the same abandoned hay culture on February 2, 1919, and were grown under similar conditions from the start (i.e., as stock cultures). No sign of conjugation was observed in either race during the first six months of their history, although frequent attempts were made to bring it about by means of isolation experiments, as well as through the experiments with salts described above. The main cultures were allowed to go dormant from about the middle of July until October 15, 1919, on which day they were renewed. On the twentieth a few conjugating pairs (three or four) were observed in *6a*, and

again on the twenty-second. Not one was observed in *5a*. Attempts were made (on October 17th and October 22nd) to induce the occurrence of conjugation in large numbers by subjecting subcultures to the action of 0.00002 N FeCl_3 and of 0.001 N NaNO_3 solutions, but the results were negative. It should be remembered, however, that these animals taken from stock cultures had been growing in a medium made up with 'tap-water,' and so perhaps might not have responded in the same way as animals in a pure aqueous medium when treated with salts. These same stock cultures were then allowed to undergo another long period of dormancy (which may be dated from November 1, 1919). Two subcultures taken from these stock cultures and renewed with spring water and hay infusion on December 12th gave, in the case of *5a*, one pair of conjugants, in *6a*, a considerable epidemic of conjugation (1 to 5 per cent) extending over a period of thirty-three days. Attempts to augment the intensity of conjugation in the latter culture by treating samples with different concentrations of FeCl_3 (0.00002 N, 0.00004 N, and 0.00008 N), with KCl (0.00025 N and 0.0005 N) and with NaNO_3 (0.001 N) were ineffective; that is, the relative number of pairs obtained in these experiments was not greater than in the renewed culture.

A quite different state of affairs was found to ensue during the later history of *17a*. As pointed out above, this race proved susceptible to conjugation during its early development. Cultures *17aI*, *II*, and *III* taken at different times from the same stock culture (which was started on January 17, 1919, from one individual) yielded conjugants very readily when renewed after two or three weeks of semistarvation (tables 1 and 2). The general effect of adding salts to the medium was to intensify conjugation in accordance with the results of Enriques and Zweibaum. After four months of cultivation, however, no more conjugants were obtained in the experiments with this race. The culture *17aV*, derived from *17aIII* (which had been continued as the stock culture), when renewed on June 28th after lying dormant for one month, gave no conjugants, and experiments with salt solutions (FeCl_3 and NaNO_3), such as were

found to be effective in earlier experiments with this race, gave negative results. A subculture, taken from the main culture *17aIII*, after it had lain dormant for two months, when renewed on September 19th, gave only one pair of conjugants. The main culture itself (*17aIII*), renewed on October 15th, after three months of dormancy, gave no conjugants, although samples were treated, as in the case of *5a* and *6a* (p. 349), with solutions of FeCl_3 (0.00002 N) and NaNO_3 (0.001 N).

Another set of experiments was conducted with these three races, after more than a year of cultivation, during the month of March, 1920. A subculture was derived from each of the stock cultures, *17aIII*, *6aI*, and *5aI*, which had been maintained in a dormant state since October, 1919, and these were renewed on March 18th with distilled water and hay infusion. A good growth of paramecia followed. Daily examination of the renewed cultures revealed no conjugants in *17aIII*, a single pair in *6aI* (on March 25th), and about 1 to 5 per cent in *5aI* between March 22nd and 27th. The race *17a* is thus shown to be non-susceptible as before, race *6a* much less susceptible than it had been during the month of December, 1919, whereas *5a* conjugated more readily than at any other time during its history. Experiments with salt solutions were found to give essentially negative results. Three experimental cultures of each race were treated respectively with NaNO_3 (0.001 N), AlCl_3 (0.00003 N), and FeCl_3 (0.00002 N) on March 23rd, and another set was treated similarly on March 26th. The treated cultures of *17aIII* gave no conjugants, and those of *6a* and *5a* no more than could be found in the renewed cultures themselves.

Parallel experiments were likewise conducted with other strains of *Par. caudatum*. In the following series of experiments seven races (other than the ones considered above) were employed (table 3). Each had been cultivated under like conditions for one month, during which time conjugation occurred in culture *2a* on November 8th (5 per cent), but not in any of the other cultures. On November 14th, 1919, a dormant culture was started from each of these continuous cultures by diluting 100 cc. of culture with an equal part of distilled water, a few pieces

of solid hay being added to each to prevent starvation of the infusoria. These seven dormant cultures were renewed with hay infusion and distilled water on March 3, 1920, changed on March 6th and March 8th. On March 9th conjugants were found in one of these renewed cultures, *2a*, to the extent of about 5 per cent; on March 12th in culture *44a*, 5 to 10 per cent; and on March 17th in culture *57a*, about 5 per cent. The animals in *44a* continued to conjugate in increasing numbers after the 12th, reaching a maximum of 30 to 50 per cent on the 15th. A similar, but less extensive, epidemic manifested itself in *57a* during a period of many days, attaining a maximum of about 10 per cent. Experiments with various salt solutions were started on March 9th, and repeated on March 12th. In the first set of experiments, in which FeCl_3 and AlCl_3 were used in the optimal concentrations recommended by Zweibaum, negative results were obtained (March 10th). In the second attempt, AlCl_3 and NaNO_3 were used, the first one in two concentrations, and this time also essentially negative results were obtained, for although conjugation was already in progress in the culture *44a*, the relative number of conjugants in the experimental cultures was not appreciably altered as a result of the treatment with salts.

If these seven cultures be compared with the continuous cultures of the same races from which they were derived originally, it will be seen that the conditions in one set tend to parallel those in the other; that is, conjugation tends to occur in two distinct cultures of the same race even after they have been subjected to quite diverse conditions. For in the continuous culture *44a* (after the dormant culture had been derived from it on November 14th) conjugation took the form of an extensive epidemic between November 16th and December 15th, and in the continuous culture *2a* conjugation occurred on November 8th (5 per cent), and again, in small numbers, on January 11, and 12, 1920. No conjugation was observed to occur in the continuous culture *57a*, but in a subculture of this race, which had been set aside on October 31st with abundant nutriment, and allowed to undergo evaporation (method of Hance, '17),

conjugation occurred to the extent of 10 to 20 per cent on November 7th, after about one-fourth of the culture liquid had evaporated. Another observation indicates that this race was very nearly as susceptible as *44a* when the conditions were not maintained uniform. Four lines of this race which had been cultivated as isolation cultures on depression slides from October, 1919, until February 6, 1920, after being merged to form a mass culture, gave conjugation on the seventh day (February 13th).

Further consideration of the development of these races from the time of their isolation is instructive, for in two of them there was observed a periodic recurrence of conjugation under more or less uniform conditions of growth. In one race, *38a*, started as an exconjugant on March 25, 1919, it occurred on May 5th (10 per cent), and in the second 'generation' (i.e., the exconjugant race *38b*, started May 5th) on July 14th, in smaller numbers (1 to 5 per cent). No subsequent epidemic of conjugation has been observed in these cultures. The two races *28a* and *39a* were started on the same day as *38a*, each from an exconjugant, and were grown under like conditions; but in neither of these races has conjugation been observed during the first four months when the cultures were regularly examined, nor subsequently when examined only at intervals. Negative results were obtained with race *39a*, as with *38b*, in the experiments referred to above.

In the other 'periodic' race referred to (*44a*, *b*, *c*, and *d*), conjugation has been repeated four times. The original culture *44a* was started from an exconjugant on September 25, 1919, and gave rise to an extensive epidemic on November 16th, which lasted until December 15th. This involved practically all individuals in the culture, for as many as 5 to 10 per cent of the paramecia were in union at any one time during most of this period. In three out of four cultures of *44b*, started from corresponding exconjugants of *44a* on November 20, 1919, a large epidemic occurred as before, between the dates January 30 and February 23, 1920. Several new cultures, started on February 2 from exconjugants of *44b*, were collectively designated as *44c*, and in two of these likewise conjugation occurred, making

its appearance on February 28th in one, on March 2nd in the other. It made its first appearance in several exconjugant strains of the fourth 'generation' (44d, isolated on February 28th from a culture of 44c) as early as the 23rd of March. In the original culture, 44a, after the occurrence of conjugation during November and December, no more conjugants were observed until March 12, 1920, when they were found to the extent of about 5 per cent. Casual pairing occurred subsequently during a period of several days.

It is important to compare the reactions of these animals of race 44 with those of race 38 during their respective periods of conjugation. In the latter strain a slight disturbance in culture conditions (addition of hay infusion) caused immediate cessation of the process, whereas in the former it exercised but a temporary and partial influence when several times repeated. We have, as it would appear, two strains in which conjugation tends to manifest itself periodically, in one of which it is largely influenced through the environment, in the other more nearly independent.

The two races 43a and 57a were derived as exconjugants from the same source as 44a, on the same day (September 25, 1919). They were cultivated under parallel conditions and examined regularly during a period of six months, but no conjugants were observed in either of these continuous cultures grown under the same conditions as 44a. During the course of the experiments described above conjugation occurred in the renewed culture of 57a, but not in as great numbers as in 44a, whereas no conjugation took place in 43a after renewal.

In the remaining race, 8a, used in these experiments, conjugation has never been observed, either in the experiments or in a continuous culture maintained under the same uniform conditions as the continuous cultures of the six other strains (listed in table 3).

It is apparent from the results of these observations and experiments with several strains of *Paramecium caudatum* that no general directions may be formulated by which we can bring about conjugation in a given strain at any one time. When

various strains are compared under the same set of conditions they respond differently: in some conjugation occurs, in others it does not. Only one of the strains (*17a*) used in my experiments may be regarded as being similar to the strain which Zweibaum employed in his studies, and it responded to experimentation only during the first five months of its history. Other strains were found to conjugate readily in cultures which were renewed with hay infusion, after they had undergone a period of dormancy, but the use of salt solutions was not found to cause any appreciable increase in the relative number of conjugants, although these same solutions had been found effective in augmenting the intensity of conjugation in *17a*.

Others, moreover, persistently failed to give conjugants under uniform and under experimental conditions. Of these, certain ones (*39a*, *43a*) were derived originally from exconjugants, and so might perhaps be regarded as conjugating strains; but the race *8a* has never been seen to conjugate, although it has been under regular observation only during a period of six months. It is possible that these strains may in time become more susceptible to conjugation. Such a condition is indicated in the two races *5a* and *6a*, in which, after a period of about eight to ten months of cultivation, conjugation was found to occur in subcultures renewed after dormancy. Previous to this time cultures had responded negatively to the same treatment.

Taking the evidence as it stands, we would be forced to conclude that some at least of the strains studied here are heritably diverse. Tested by the experimental methods of Zweibaum, which he claims would be effective for any given race of *P. caudatum*, I have found consistent diversities between races as regards conjugation. My observations upon 'periodic' races, moreover, support this same general conclusion. It would be possible to point out other differences between the various races which I have studied, e.g., as regards size, shape, division-rate, etc., but since we are concerned here mainly with differences in respect to conjugation, I shall not attempt any such comparisons. Differences in respect to size and fission-rate have been studied in other races by Jennings and Hargitt ('10), but accord-

ing to Zweibaum these supposed racial diversities result from differences in the amount of nutriment. Supposing, however, that acquired diversities may arise in different cultures as a result of differences in environment, these same cultures should, in Zweibaum's opinion, yield conjugants after being subjected to the prolonged experimental procedure which he recommends. But this they fail to do in all cases, as the results of my experiments indicate. Cultures which are known to have a common ancestry (various cultures of the same pedigreed stock, descended from one original individual) do respond similarly in these experiments. But when races of diverse ancestry are compared they almost invariably respond differently. Two races coming from the same habitat (as from the same pond or stream, aquarium, or hay culture) may sometimes respond similarly under the same conditions, but as a rule they respond differently. Thus, the two races 38 (*a* and *b*) and 39*a* were from the same general source, a pond near Baltimore, but the first underwent two epidemics of conjugation in the laboratory, during which time no conjugation has been observed in 39*a*. Similarly, the two races 44*a* and 43*a* were isolated from the same lot of material collected in a river. In 44*a* (and its derived cultures) conjugation has occurred in epidemics of long duration under essentially uniform conditions, whereas in the latter strain (43*a*) no conjugation has been observed since the beginning, under uniform conditions or under the altered conditions of growth which favored conjugation in 44*a* (as when cultures are renewed after dormancy).

Summary of observations and experiments on Paramecium caudatum. 1. There are diversities among the various strains of this species as regards their tendency to conjugate under natural and experimental conditions.

2. Some of these diversities in response under a given set of conditions may be qualitative, as illustrated by the different behavior of two conjugating strains when treated with a particular reagent. That is, the conditions for conjugation in all strains are not identical.

3. That the several strains in question are racially diverse is supported by the fact that conjugating strains sometimes

maintain their ability to conjugate freely for several 'generations,' in new cultures started from exconjugants of a previous epidemic.

b. Paramecium aurelia

My studies upon races of this species open up problems essentially like those presented by *caudatum*. Some of the cultures were subjected to daily observation during the first weeks of their development. Conjugation was thus shown to be of frequent periodic occurrence in some, rare and apparently sporadic in others, while in some it was found neither during this period of daily observation nor on subsequent examination at intervals of two to five days for several months. At intervals dense watch-glass cultures, derived from the main cultures, were set aside in moist chambers and examined the following day for conjugating pairs. In such cultures the nutriment is quickly used up by the paramecia, and the conditions are right for an epidemic of conjugation, provided the organisms were taken from a flourishing culture (method of Maupas, '89). In this manner some of the races which showed no conjugants in the stock cultures were made to give several in these isolation experiments, while others, which showed small numbers in the stock cultures, yielded as many as 50 per cent in the experiments. As a rule, pairing took place earlier and persisted later during a series of such experiments than in the main cultures themselves, when the experiments were begun sufficiently in advance of an epidemic of conjugation in the main culture. That is, conjugation could generally be obtained in the dense watch-glass cultures several days before any pairs were seen in the stock culture from which they had been derived, and could be obtained for days after it had ceased in the stock culture. The effect in such experiments is, then to augment conjugation in cultures of organisms already in a state of susceptibility. Certain races, however, failed to conjugate during a period of several months, even when placed under the more favorable conditions mentioned above (isolation experiments). In this connection, also, solutions of various salts were added to some of the watch-glass cultures when isolated. This served to increase somewhat

the percentage of the conjugants in certain more susceptible races, but many that did not conjugate still remained.

From the above it will be seen that among the various strains of *Paramecium aurelia* there appear to be several intergrading conditions as regards conjugation; in some races it occurs spontaneously, in others its occurrence is conditioned (in the laboratory) by certain experimental proceedings—alterations in environmental conditions. The question arises whether we cannot by still other, perhaps more indirect, methods bring about conjugation in all races of this species. So far as the results of my work go, the question may be answered in the affirmative. Of the thirteen races of *P. aurelia* studied in the beginning, all proved amenable to indirect methods. Six were found to be conjugating races at one time during their existence; that is, they conjugated under more or less uniform conditions of cultivation. Seven were found to be more refractory, yielding only to indirect methods.

The most effective procedure for these particular races was to subject them to a period of semistarvation lasting from four to eight weeks, then to bring each to a state of rapid multiplication by adding hay infusion containing certain electrolytes to the culture. The culture medium used most effectively contained NaNO_3 , final concentration about 0.001 to 0.004 N, and a smaller proportion of $\text{Ca}(\text{NO}_3)_2$, (0.0001 to 0.0004 N). The solution used in later experiments contained NaNO_3 alone, and this gave about as good results. It was thought that the calcium used in these preliminary experiments would offset the toxic effect of sodium, and so lead to better growth in the culture, but it was found that the sodium salt when used in low concentrations (0.001 to 0.003 N) was relatively non-toxic to most races of *P. aurelia*. As will be shown later, the effect of sodium salt upon *Paramecium* is to stimulate growth and thereby accelerate the fission-rate.

The period of semistarvation to which it was found necessary to subject each culture differed considerably, depending upon the race employed. Some few of these (14a, table 5, and 16a) proved to be very refractory, for they failed to give conjugants

except after fasting for eight to nine weeks. Others, on the other hand (e. g., *13a*, table 4, and *25a*), conjugated after shorter periods of dormancy—about three to four weeks. The duration of this period serves perhaps as the best criterion for judging the relative susceptibility of various races toward conjugation, for experiments with salt solutions generally give negative results unless the organisms have become susceptible to conjugation previously through dormancy.

The effects produced by using salt solutions directly in experiments designed to induce conjugation (method of Zweibaum) are rather variable and unpredictable. As a rule, the salt solutions increase the percentage of conjugating pairs, but I suspect that their action is largely osmotic rather than chemical. When added to the main cultures when renewed, however, the effect of sodium salt is more marked, although even by this method a relatively small number of conjugants is obtained. As examples of this effect may be cited experiments with race *13a*, table 4; *14a*, table 5; *11i* and *11j*, table 6, and *3d*, table 7. The reactions of these various races, however, may be contrasted with that of race *1b*, table 7, in which conjugation is not favored by the presence of sodium nitrate, or, if so, only by a lower concentration than that which was found most effective in the other races.

In order to bring out more clearly some of the racial differences between the various strains of *P. aurelia*, the following observations are recorded. They indicate, also, something regarding the nature of the 'periodicities' in conjugation so characteristic of many strains of this species.

Par. aurelia, *1* (*b* and *c*). This race was descended from a single individual isolated January 30, 1919, from an old laboratory hay culture. The original culture, *1b*, developed very rapidly under laboratory conditions, the animals proving to be quite hardy at all times during their history.

A watch-glass culture containing numerous individuals was set aside on March 17th, and on the following day showed conjugation involving about 10 per cent of all. Observations failed to reveal the occurrence of the process in the stock culture

previous to the 19th, when one or two pairs were noted. It was recorded at intervals during March and April, but on each occasion in very small numbers (involving usually less than 1 per cent). During a period of thirty-two days (March 17th until April 18th) very frequent recourse was had to isolation experiments, in watch-glass cultures, in order to determine whether the organisms continued susceptible. Every one of these experiments yielded conjugants, in numbers ranging from about 5 per cent to as high as 50 or 60 per cent in some instances. Throughout most of this period no conjugation was noted in the stock culture, either on the day preceding each isolation experiment or on the same day. It frequently happened that in a dense culture set aside in a watch-glass conjugation set in almost immediately, so that at the end of one-half hour 10 per cent of the paramecia would be found in union, although no pairs had been seen in the beginning. Other races responded similarly in these experiments, but not so quickly nor in such large numbers.

The strain *1b* was subjected to experimentation on September 30, 1919, when renewed cultures were started in small Stender dishes with material taken from the stock culture. This had been allowed to stand for over three months without renewal, although considerable hay was added to it previously. The animals were found to be in a very healthy state, showing no effects of starvation, although they were not dividing. Practically no conjugants had been observed in the culture since May, 1919, when casually examined. On the second day after renewal conjugation was obtained (on October 2nd) in the culture *1b-A* to which no electrolytes had been added. In the other cultures, containing NaNO_3 , pairing took place in smaller numbers (table 7).

As was pointed out above (p. 358), the reaction of these organisms to the presence of NaNO_3 in the renewed culture is in marked contrast with that of the other races to be described later, for which a low concentration of this salt acts as a stimulant to conjugation. The animals of culture *1b* (and of *1c*) always conjugated most readily in an aqueous medium, whenever

they conjugated at all. In the stock cultures the paramecia conjugated little or not at all, but they remained susceptible during long periods of time, yielding conjugants readily in watch-glass cultures isolated from the main cultures.

Par. aurelia, *3a*. The culture was started January 23, 1919, from an individual isolated from the same abandoned hay culture as *1b*, and so it is not known with certainty whether it represents a race distinct in descent from *1b*, although its characteristics are sufficiently distinct to render this probable. Conjugation was observed in this culture while still small on February 13th, in about 20 to 30 per cent of all, and again on February 15th and 17th in smaller numbers (3 to 5 per cent). Conjugants were again seen in this culture on March 12th, about 1 per cent, and later between March 17th and 22nd, in no case in more than casual numbers. Isolation experiments continued to yield them, however, often as many as 5 per cent, from March 15th until April 18th, when the experiments were discontinued.

Culture *3b* was derived from an exconjugant of *3a* isolated on February 13th. One or two pairs of conjugants were seen in the culture on April 7th, after long search, while isolation experiments yielded them in small numbers (1 per cent or less) between March 19th and April 10th. Except for the casual pairs noted above (April 7th), the stock culture showed none from its beginning until the time when regular observations were discontinued.

Cultures *3c*, *3d*, and *3e* were started on March 20th from three exconjugants of *3b*, and represent thus the third 'sexual generation' of the race. Each was cultivated under uniform conditions and observed regularly. Conjugation occurred to the extent of 40 per cent in a watch-glass culture derived from *3d* on April 16th, but was not discovered in the main culture during the entire period of daily observation (from March 20th to April 20th), nor on subsequent examination at longer intervals. Isolation experiments set before and after the 16th (on April 15th, 17th, and 18th) gave no conjugants. The two related cultures, *3c* and *3e*, failed to show any conjugation, even in the isolation experiments, which were continued until April 20th.

Comparing this race, no. 3, with race 1, it will be seen that in both conjugation occurred naturally under the more or less uniform conditions afforded in large stock cultures. In culture 3*a* there is at first apparently a periodicity marked by 'epidemics' in the occurrence of the process at intervals of about four weeks. In culture 3*b*, started from an exconjugant, the first epidemic appeared after about five weeks, and in 3*d*, similarly related to 3*b*, after about four weeks. Conjugation in race 1*b*, while not occurring at periodic intervals in the stock culture, was readily evoked in isolation experiments during the entire period when such experiments were regularly conducted (March 18th to April 18th). The same irregularity tends to manifest itself in the culture 3*a* after the onset of the second period of conjugation (March 12th).

This irregularity in the occurrence of conjugation during the later stages of growth in a culture in which it has already taken place, in the form of a preceding 'epidemic,' makes it impossible to measure accurately the interval between conjugation periods except with cultures started from exconjugants, and in which conjugation has not previously occurred. Even the first epidemic of conjugation in a newly developed strain may show two separate phases (see observations on races 11*c* and 11*f*, p. 363) suggesting that diverse lines, showing acquired differences in susceptibility as regards conjugation may have arisen among the offspring of one individual. Later irregularities in conjugation—after a previous epidemic in the same culture—may perhaps be accounted for in part, also, as due to hereditary differences established through conjugation, among the exconjugants of the first epidemic. Thus diverse strains, with different degrees of susceptibility, each having perhaps a somewhat different periodicity, might be perpetuated in the same culture coming originally from the same race. If this be true, a new clone derived from a single exconjugant would presumably give conjugants in a more sharply defined epidemic than a culture containing the offspring of several exconjugants, although originally from the same strain.

Some such interpretation as this may account for the irregular occurrence of the process in the later history of the mass culture *3a*, for a study of the later generations (*3b* and *3d*) shows that conjugation in these, like the first epidemic in *3a*, is sharply defined. Although no sufficient evidence is here afforded that hereditary differences exist between the corresponding cultures of the same generation of exconjugants (*3c*, *3d*, and *3e*) in respect to conjugation, some observations made upon exconjugant lines of the race *44* (*P. caudatum*) indicate that such heritable diversities do arise in this manner (p. 373).

P. aurelia, *11a*, was obtained as a mass culture on February 15, 1919, from Professor Jennings, by whom the race had been reared from one individual isolated January 18, 1919. Conjugation had already been observed in this culture on February 5th, and again on February 15th, involving at each epidemic a fair percentage of individuals. On March 5th and 8th pairing was observed in about 10 per cent of all, and from March 10th to 12th its occurrence was noted as casual. Conjugants were seen again, in smaller numbers (about 3 per cent), on April 4th, although casual pairs were found as early as March 29th, and later, on April 9th. The process, it will be seen, is becoming more irregular in occurrence as time progresses, the separate epidemics becoming much less distinct and the percentage obtained at any one time falling off. This is evidenced, also, by the fact that, although conjugants were not discovered in the main culture between March 12th and 29th, yet isolation experiments continued to yield them in considerable numbers (5 to 30 per cent) between these dates. Three, and possibly four, successive epidemics are thus indicated for race *11a*. Results of later observations (in races *11c* and *11f*, p. 363) showed that the occurrence of two sharp epidemics close together in point of time, such as those of February 5th and February 15th, or March 5th and March 8th, may very properly be regarded as one, the first onset being interrupted by an abrupt change in nutritive conditions (the addition of hay infusion to the culture, etc.).

Cultures *11b*, *11c*, *11d*, and *11f*. These were derived from corresponding exconjugants of *11a* isolated on March 20th. A well-marked epidemic of conjugation, involving about three-fourths of the culture, occurred in *11c* on April 4th. The process was repeated on April 14th (about 5 per cent), and continued until April 18th (casual). In culture *11f* conjugation appeared first on April 15th (casual), and on the day following, to the extent of about 60 per cent. On April 17th about 10 per cent were in union. No conjugants were observed in *11b* and *11d*, examined daily until April 18th, and casually thereafter.

Exconjugant strains of *11c* were started from ten pairs isolated April 4th, and of *11f*, from six pairs, on April 17th. The twenty exconjugant cultures of *11c*, designated as races *11g* (1 to 20), were grown under very uniform conditions, in hay infusion made up with distilled water, and renewed each week. Under the favorable conditions thus imposed, no conjugation was observed in any of the cultures for over two months. Similar negative results were obtained with the twelve strains derived from *11f* exconjugants, designated as *11h* (1 to 12), subjected to daily observation for six weeks and examined casually afterward.

On the 6th of July an individual of *11g* (from a culture which had lain dormant for about a month) was isolated in a watch-glass, in hay infusion containing 0.003 N NaNO_3 . On July 10th conjugation occurred among the numerous progeny to the extent of 15 per cent. From the exconjugants two daughter strains were started (*11i* and *11j*). No conjugation was noted in these cultures prior to July 24th, when they were left unobserved and unaltered.

In each of these conjugation was again obtained in small numbers during September by renewing the dormant cultures with hay infusion containing NaNO_3 (table 6). No conjugation occurred, however, in those cultures which were renewed with hay infusion alone.

The study of *11a* and its descendants reveals a condition which is prevalent in various other races of this species (e.g., *3a* and *23a*), as well as in *caudatum*. Conjugation was found to occur very readily in cultures of the race during the first

few months of its history, usually under fairly uniform conditions; whereas later the process could be evoked only after renewing cultures which had undergone dormancy. The natural periodicity which characterized the early stages became suppressed, and the relative number of conjugants obtained likewise fell off considerably as time progressed. The effect is probably not due to unfavorable culture conditions long maintained, although this may play some part, nor to close inbreeding; but is probably connected in some way with the adjustment of the organisms to a state of continuous, rapid multiplication. This point will be treated more at length in part 2 of this paper (p. 371).

The eight races designated *13a*, *14a*, *15a*, *16a*, and *23a*, *24a*, *25a*, and *26a* were derived on February 28, 1919, from the same source—an abandoned hay culture containing various infusoria. Each was descended from a single exconjugant, the original individuals of *13a* and *23a* having come from the two members of one pair, *14a* and *24a* from another pair, etc. It is important to compare these races from the start, since all had been subjected to the same environmental influences previously. The cultures were examined frequently for eight weeks, while numerous tests for conjugation were made with dense watch-glass cultures isolated from the main jars. All the stock cultures developed rapidly, except *24a* and *26a*, in which growth was more tardy, owing in part to overnutrition, to some extent possibly to racial weaknesses. Conjugation occurred in two cultures, *23a* and *25a*, during the period of continuous cultivation, but not in the six others.

In *23a* several pairs were observed as early as March 11th, eleven days after the previous union, and again on March 17th. They were found in larger numbers (5 to 15 per cent) from March 18th to 22nd, but only casually thereafter until March 31st, when conjugation practically ceased in the continuous culture. Isolation experiments gave as high as 40 per cent on March 15th, and subsequently until April, in diminishing numbers (March 18th, 40 per cent; March 20th, 30 per cent; March 26th, 5 per cent, etc.).

The epidemic in *25a* was less intense, involving at any one time a much smaller percentage of organisms, and lasting but a short time (Mar. 18th to 25th). Isolation experiments yielded conjugants during a somewhat longer period, but in scarcely any greater numbers.

The sequence of events in these two cultures is seen to be very similar to that which obtains in such races as *3a* and *b*, *11a* and its derivatives. That is, there is a period of vegetative growth lasting from two to three weeks, after which conjugation ensues. The duration of this period of conjugation depends in large measure upon the susceptibility of the races in question, beginning earlier and lasting longer in the more susceptible ones—except in some few instances where, in small cultures, the process is consummated in one or two days (e.g., *11f*).

In order to determine whether these races (*23a* and *25a*) which had conjugated under uniform conditions had really done so as a result of their greater natural susceptibility, and not, as might be supposed, because of more favorable environmental conditions, new strains were started from isolated exconjugants of each. In this way the initial medium was greatly altered, new strains of bacteria coming in, with the elimination perhaps of many strains of bacteria present in the old cultures. Of the two strains derived from exconjugants of *23a* which were reared successfully, conjugation occurred in each, approximately three weeks later. In one of these, *23f*, isolated March 21st, conjugants were observed on April 14th (5 to 10 per cent), April 15th and 16th (casual). In the other culture, *23i*, isolated March 21st, conjugation was only casual, one pair being seen on April 12th in a mixed culture of two races, *P. caudatum 17a* and *P. aurelia 23i* (table 2). Two cultures of *25a* exconjugants (*25e* and *25h*) started on the same day as those from *23a*, and similarly treated, showed no conjugants, even in isolation experiments. The relative susceptibility of these two races, of the second generation, to each other is thus shown to be the same as for the two parental strains. That is, the race *23* seems more susceptible than *25* in each case, although conjugation failed to

occur in the second generation of 25, and was less intense during the second epidemic (second generation) of 23 than during the first.

In the six remaining stock cultures (of races 13a, 14a, etc.) no conjugation was found to occur during the first five months while continuous cultivation was resorted to, although it was obtained by indirect (experimental) methods. Those experiments in which salt solutions were used in the way recommended by Zweibaum gave less striking results in this species than in the one caudatum race, 17a, in which they were effective. Compare, for example, the results of such experiments with race 13a (*P. aurelia*) with those in race 17a (*P. caudatum*) when both strains were growing in the same culture (table 1). As was pointed out on page 358, cultures of nearly all races, when renewed with hay infusion containing NaNO_3 (0.001–0.004 N) may give conjugants when none can be found in controls, renewed with an aqueous medium.

The duration of dormancy preceding the growth period is perhaps the most potent influence favoring conjugation, for it was only by renewing cultures which had long been dormant that conjugation could be elicited at all in such races as 14a (table 5), and 16a. In these two strains a preliminary period of dormancy of seven to eight weeks was required before conjugation could be obtained in the cultures renewed with a saline medium. With other races, e.g., 24a and 25a, only three to four weeks of dormancy were required before renewal in the same way. One of these (25a), it will be noted, had been found earlier to conjugate under uniform conditions (in the stock culture), although it had later lost this tendency. It was shown afterward that three months of dormancy was sufficient for conjugation in any of the races of *aurelia* when renewed with an aqueous medium (tap-water), for when the stock cultures were renewed in October, 1919, after this period of dormancy, a few conjugants were seen in all of those cultures in which none had been discovered previously, as well as those in which they had been found before.

Summary of observations and experiments on Paramecium aurelia. 1. In this species, as in the preceding, there are diversities in respect to conjugation. Some strains show a marked tendency to conjugate repeatedly at regular intervals, while others, under the same uniform conditions, do not conjugate during long periods of time. Diversities in response to the same set of experimental conditions are sometimes indicated, as when one race shows a greater tendency to conjugate in a particular saline medium than in an aqueous medium, another race so treated showing a lesser tendency.

2. These diversities probably have a racial basis, for if two cultures of the same strain, each having had a similar history, be subjected to the same treatment they show about an equal tendency to conjugate. Furthermore, when two different strains are compared they are found to preserve about the same relative degree of susceptibility, under the given set of conditions, as they showed in the beginning.

Comparing, now, these two species of *Paramecium* as regards conjugation, we find certain points of resemblance as well as differences. They each respond, in general, to the same set of experimental conditions—dormancy, renewed division, etc.—and show some similarity in their behavior when treated with salts. In each species there is a tendency for conjugation to occur periodically in some strains, although the intervals between periods are not of like duration. In the smaller species (*P. aurelia*) the life processes appear to be contracted into a shorter space of time than in the larger, but the same kind of internal factors seem to be operative in both species.

The rôle which these internal factors play is best shown when we subject a strain to a uniform environment, or by testing experimentally (using the same experiment each time) at frequent intervals during a period of several months. Studied in this way, the organisms show periods of greatest susceptibility and refractory intervals during which conjugation does not occur.

On the other hand, there is reason for believing that the internal factors may be slowly modified with time, perhaps by the prolonged adjustment of the organisms to a new environment. Strains which early in their history showed a marked tendency to conjugate periodically lose this power as time progresses. Evidence bearing on this point has been presented: for *Par. caudatum*, race 17*a* on page 349; race 38, page 352, and for *Par. aurelia*, race 3, page 360; race 11, page 362, races 23 and 25, page 365. Those races which, early in their history, showed a tendency to conjugate at frequent intervals, and in large numbers at each epidemic, retain their susceptibility longer than those races which at first conjugated only at longer intervals and in smaller numbers. The susceptibility of such cultures in which conjugation is in abeyance can often be partially restored by subjecting them to a long period of dormancy. Thus, a culture of *Par. aurelia*, 23*i*, renewed on January 10, 1920, after a five-month period of dormancy, gave conjugants on January 16th. From an exconjugant a new culture, 23*k*, was started, and in this conjugation was repeated under essentially uniform conditions. (In several new exconjugant lines, 23*l*, (1) to (6), however, conjugation did not occur.) This shows that the very long dormant period to which the organisms of this race were exposed had restored their power to conjugate periodically, as in the original culture, 23*a*, of this race (p. 364).

2. GROWTH FACTORS INFLUENCING CONJUGATION

On the basis of the above observations, and by the aid of supplementary experiments, I shall attempt to analyze the conditions which are known to influence the occurrence of conjugation in *Paramecium*. Three outstanding factors or relationships call for explanation. These are, 1) the relation of fission-rate to the onset of conjugation; 2) the state of dormancy, and, 3) the periodicity which characterizes freely conjugating strains.

A comparative study of division-rate as such, in the various races of *Paramecium* here treated, shows that there is no simple correlation between high or low division-rate and a strong tendency to conjugate. Among those races having a high rate of

fission, and resembling each other closely in size and appearance, there are those which conjugate repeatedly and those which do not, and the same may be said for the slowly dividing strains.

But it seems probable, in the light of our experimental knowledge, that there is an indirect connection between conjugation and fission-rate. Whenever conjugation occurs in a culture en masse, it usually does so after a period of accelerated division. This relation has been emphasized by several investigators, working with diverse genera of Infusoria, both free living and parasitic. In fact, Maupas regarded it as one of the primary conditions preceding a conjugation epidemic in the many species which he studied, and applied it practically in his work.

In further support of this view—that conjugation is conditioned by a preliminary period of increased division—we may recall the effects produced by adding salts to the cultures of *Par. aurelia* when renewed. In a medium of sodium nitrate (of 0.001 to 0.005M concentration) conjugation is almost invariably augmented in this species (cf., however, race *1b* to the contrary, which conjugated as well in an aqueous medium). Oftentimes conjugation can be elicited in such 'salt cultures' when none occurs in the controls renewed with an aqueous medium. It may be shown very easily by means of slide cultures, changed daily, that NaNO_3 in such concentrations acts as a stimulant to growth. The division rate in these treated lines may be maintained at a considerably higher level than that of the controls for at least two weeks (table 8). Some races apparently do not tolerate the continuous action of the salt and die after two or three days of accelerated growth, and the death rate is greater in any race so treated than in the controls. These observations apply to races of both species of *Paramecium*, but only occasionally is a race of *caudatum* found which will tolerate the action of the salt as used in these experiments.

J. Spek ('19) has conducted similar experiments on *Par. caudatum*, using various salts in the medium, and finds that many of these tend to accelerate fission-rate.

My experiments upon *Paramecium* using MgSO_4 in the culture medium indicate that this salt has a depressing effect upon

growth. Most races become readily adapted to a 0.01M concentration, and may continue to live for months in such cultures to which hay has been added. The division rate is not appreciably diminished by this salt, although extensive experiments may yield more definite results than I have thus far attained. In the few conjugation experiments in which MgSO_4 was used (*P. aurelia*) the results were negative, except when the solution was used indirectly to increase the osmotic concentration of the medium in a derived culture. These cultures, when renewed with such a medium, continued to live, but failed to grow with sufficient rapidity to develop the initial impulse to conjugate (ex hypothesi).

Let us now consider the second condition, or antecedent, to conjugation, namely, that of subjecting the cultures to a period of dormancy. From what we know concerning the relation of fission-rate to conjugation, it might be conjectured that such a period of rest (dormancy) renders the organisms so treated potentially more capable of rapid division than those which had been dividing previously. Or, conversely stated, a regulation in division-rate may occur in organisms subjected to long-continued multiplication, so that they do not divide so fast as formerly. Such a regulation has been shown by Jollos ('13) to occur in paramecia subjected to a higher temperature. Lines which had been growing at 19° , after being transferred to 31° , divided at a rate of 9 per forty-eight-hour period; after six months at this temperature, however, at the rate of 7.

If any regulation in division-rate does occur in organisms subjected to nutritive conditions favoring rapid growth, one ought to be able to test it experimentally, using two cultures of the same strain, one of which had lain dormant for several months, the other having undergone continuous growth. Accordingly, such an experiment was tried. A race of organisms (*P. caudatum* 39) in which conjugation had never been observed was chosen in order to eliminate any secondary effects which might accompany this process. The culture 39A had been maintained under rather uniform conditions of growth, renewed with hay infusion every two or three weeks for a period of about six months,

during which period the culture 39a had lain dormant. Both cultures were examined daily during the two weeks preceding the experiment, but showed no conjugants. The mean division-rate in fifty-two lines from 39A was found to be $11.25 \pm .175$ for a twelve-day period; the mean rate in forty-four lines from 39a was $15.70 \pm .193$ for the same twelve-day period. The effect of long-deferred division, i.e., dormancy, is, then, to render the organisms capable of more rapid division when favorable growth conditions are afforded. It follows from these results that dormancy may influence conjugation through its indirect effect on fission-rate, if it be true that rapid division induces conjugation. This may serve to explain, also, why it is that conjugation occurs most readily in cultures of infusoria which have but recently been brought into the laboratory and subjected to conditions producing rapid growth. For obviously the conditions in ponds and streams are not ordinarily such as to maintain these animals in constant division, as we find in a fermenting hay culture, so that when brought into the laboratory they are in essentially a dormant condition, and will multiply rapidly when the conditions are right. On the other hand, the tendency for conjugation to become suppressed in previously conjugating strains, after prolonged cultivation (p. 368), receives an explanation: it is a phenomenon accompanying the gradual loss of the power of division.

A third line of evidence favoring the view that conjugation is initiated by a period of unregulated division is obtained from the records of fission-rate in strains which conjugate at regular intervals. As pointed out by Hertwig ('89) and fully demonstrated by Jennings ('13), the most apparent immediate effect, produced by conjugation is a decrease in fission-rate. This lowering tends to disappear completely in about two months (*P. caudatum*). My observations on conjugating strains indicate a similar condition which may be repeated after each recurrence of the process; and this has led me to think that the periodicity in conjugation may in some way be controlled by this tendency for fission-rate to increase gradually to a high point. This is the essential idea involved in Hertwig's explanation of conjuga-

tion ('89) as a process following a period of unregulated division, although he recognized also an alternative process of nuclear reorganization ('parthenogenesis') in *Par. aurelia*. He says (p. 226):

Für das, was ich beweisen möchte, genügen die Resultate in ihrer jetzigen aphoristischen Form; sie zeigen, dass fortgesetzte Theilungen im Infusor eine Neigung zur Conjugation veranlassen, dass aber bei künstlicher Verhinderung der Vereinigung die Theilungen in lebhaftester Weise fortgesetzt werden, bis ein Moment eintritt, in welchem ohne Conjugation ein Ersatz des Hauptkerns durch Abkömmlinge des Nebenkerns eintritt.

Wer die Conjugation der Infusorien erklären will, muss dem Gesagten zufolge mit 2 Erscheinungen rechnen: 1. fortgesetzte Theilungen ohne Conjugation führen zum Untergang; 2. trotzdem besitzt das Infusor zur Zeit der Conjugation unverminderte oder sogar erhöhte Theilfähigkeit. Wenn wir Bütschli's Theorie zu Grunde legen, stehen beide Sätze miteinander im Widerspruche; sie sind aber sofort in Einklang zu bringen, wenn wir annehmen, dass zur Zeit, wo die Conjugation eintritt, nicht eine herabgesetzte, sondern eine übermässig erhöhte Lebensenergie besteht. Dann hat die Conjugation nicht den Zweck, die Lebensenergie noch weiter zu steigern, sondern die gesteigerte Lebensthätigkeit so zu regulieren, dass sie nicht zur Zerstörung des Organismus führt; sie heilt nicht die durch physiologische Usur entstandenen Defecte, sondern verhindert, dass derartige Defecte durch Uebermass der Function entstehen.

His later observations ('04) on *Dileptus* are likewise in accord with this earlier expressed view.

The following observations, which were made at various times during the course of my work, have a bearing upon this point. In the race 44a, of *Par. caudatum*, started September 25, 1919, from an exconjugant; the fission-rate was found to attain to a maximum during the period from November 15th to November 28th (eighth and ninth weeks) in four lines started in October. And it was at this time, also, that conjugation was extensive in the stock culture of 44a, commencing on November 16th and lasting until December 15th (eighth to eleventh weeks). From exconjugants of this culture sixteen new lines were started—44b (1) to (8), two lines from each. Both lines of 44b (5) died after six weeks, but from the remaining strains, four mass cultures were derived: 44b (2) and (3) on January 9th, 44b (7), and (8) on January 21, 1920. Conjugation occurred extensively in

three of these mass cultures, during approximately the same period of time in each, beginning on January 30th in 44b (7) and lasting until February 27th. Just preceding conjugation, during the tenth week, the fission-rate in the slide cultures reached the highest point which it had attained since the second week following the previous conjugation, as shown by the average number of divisions weekly for all the lines. (This first maximum may possibly represent a compensatory tendency after the drop immediately following conjugation, for the average rate for the first two weeks is less than that for the second two weeks.) New strains were again started from exconjugants of the culture 44b (2) on February 2, 1920, and were designated 44c (1) to (12). From the six which survived, mass cultures were started on February 16th, and in two of these conjugation set in at the end of the fourth week. The fission-rate for these two strains attained its maximum during the fifth week. A similar set of experiments was conducted, using forty exconjugant strains derived from one of the above cultures, and designated 44d (1) to (40). Twenty mass cultures were started, and in six of these conjugation occurred, setting in during the fourth week in each one. The fission-rate (in four lines of each strain) attained its highest point at about the third week. It is significant in this connection to note that in almost every instance conjugation occurred in rapidly dividing strains. Slow strains, although derived originally from exconjugants of the same race, do not conjugate, even after a period of three months has elapsed.

These experiments bring out another point which has already been touched upon (p. 362). There appear to be racial diversities among the exconjugant strains, originally from the same race. When a particular strain shows a tendency to undergo depression, all of its four lines may be affected, and frequently all die at about the same time (usually at the end of four to six weeks), whereas in the four lines of some other strain no depression may occur at this time.

My experiments with *Par. aurelia* likewise support the view set forth above: that conjugation occurs at about the time

when division is at its maximum or shortly afterward. Thus, in the strains of *23k* (p. 368) the fission-rate was highest during the second week, and conjugation occurred (in one culture) during the third week.

All this lends support to the hypothesis that it is the unusual conditions of rapid growth which predispose to conjugation. Circumstances which favor rapid growth may likewise favor conjugation. Abundant nutriment and the presence of reagents which stimulate growth without harming the organisms act in this way, but for these conditions to operate most effectively the organisms themselves must have come from freely conjugating strains, rendered potentially more capable of rapid division through dormancy.

GENERAL SUMMARY

The following conclusions regarding the causes and periodicity of conjugation in *Paramecium* may be drawn from the observations and experiments above set forth.

1. Various strains of the same species display different degrees of susceptibility, or readiness, to conjugate when taken from a similar environment and grown under like conditions.

2. They may likewise show diverse reactions as regards conjugation when subjected to the same experimental conditions, as when treated with a certain salt solution.

3. A high degree of susceptibility is indicated by a tendency for conjugation to appear in successive 'epidemics' within a given strain and by the relative number of individuals involved.

4. The observed time interval between conjugation periods in *Paramecium caudatum* is from four to twelve weeks. Conjugation was recurrent in culture *38a* after six weeks, in *38b* after ten weeks. In culture *44a* it occurred after seven to eight weeks, and again after twelve weeks, but in the smaller cultures derived from it (*44b*, *c* and *d*) at intervals of about four to eight weeks.

5. The time interval in *Paramecium aurelia* is from two to six weeks (pp. 361 and 365).

6. There exist strains which do not conjugate during long periods of time, and in which, were the conditions maintained

uniform and favorable for growth, the process might probably be postponed indefinitely (cf. Woodruff's culture).

7. Many of these races which do not conjugate can be rendered susceptible to conjugation by subjecting them to a prolonged period of reduced nutrition, thus inhibiting multiplication. Such 'dormant' cultures, of any race, when renewed by supplying abundant nutriment, may yield conjugants more readily (if conjugation occurs at all) than control cultures of the same strain which have been kept under conditions of constant growth.

8. The addition of certain salt solutions to samples taken from these renewed cultures after a few days of growth may give an increase in the relative number of conjugants in certain races, without affecting appreciably the proportion of conjugants in other races of the same species (as worked out especially for *Par. caudatum*).

9. A small amount of sodium nitrate added to the renewed cultures at the time of their renewal, serves to augment conjugation in many races of *Par. aurelia*. Diverse races appear to react differently toward equal concentrations of the salt as used in this way, for race *1b* and its derivatives underwent conjugation more readily in aqueous medium than in the solutions of the salt in concentrations found most effective for the other races of this species. This same race multiplied more steadily and maintained its numbers longer in aqueous cultures than other races of this species.

10. There is a progressive tendency, probably in all races, for conjugation to become suppressed after prolonged cultivation under laboratory conditions (p. 368). The susceptibility of such cultures in which conjugation is in abeyance can often be partially restored by subjecting them to a long period of dormancy, as described under point 7.

11. This restored power of conjugation in organisms subjected to long dormancy is correlated with their higher rate of fission, as compared with those organisms of the same race which have previously been undergoing division.

12. From what is known regarding the effects of salts in accelerating fission and in augmenting conjugation, it appears that conjugation is initiated by a period of unregulated division.

13. The periodic occurrence of conjugation in certain strains of *Paramecium* may likewise be brought into relation with periodic changes in the division-rate of these strains.

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EXPLANATION OF TABLES

In the tables which follow (1 to 7, inclusive) are recorded the results of experiments designed to induce conjugation in the various races of *Paramecium* (*aurelia* and *caudatum*). The tables indicate how long each culture was subjected to dormancy, when renewed, and the nature of the culture medium. Distilled water (and hay infusion) was used except as otherwise noted. The concentrations of 'salt cultures' are expressed as final concentrations for the salts in question.

Conjugation is expressed, when absent as 'none;' when present, in terms of the percentage involved (approximate). 'Casual' conjugation is about 1 per cent or less, or an indefinite small number.

Unless otherwise stated, the experiments with salts (and other reagents), given in the last column of some of the tables, were conducted, using one part of culture containing numerous paramecia and three parts of the reagent.

TABLE 1

DORMANT CULTURE	DATE RENEWED; CULTURE MEDIUM	CONJUGATION IN RENEWED CULTURE	SUBCULTURES 24 HOURS AFTER TREATMENT: CONJUGATION
P. caud. 5aII, March 28, 1919	A. April 11 (aqueous)	None	April 18, untreated None
	B. April 23 (aqueous)	None	April 28, untreated May 3, + NaNO ₃ (0.001N) May 3, + FeCl ₃ (0.00002N) None None
	C. May 9 (aqueous)	None	May 14, + NaNO ₃ (0.001N) May 14, + FeCl ₃ (0.00002N) None None
	D. May 28 (aqueous)	None	May 26, + NaNO ₃ (0.001N) May 28, + NaNO ₃ (0.001N) May 26, + FeCl ₃ (0.00002N) May 28, + FeCl ₃ (0.00002N) None None None None
	A. April 11 (aqueous)	None	April 14, untreated April 18, untreated None None
	B. April 23 (aqueous)	None	April 28, untreated May 3, + NaNO ₃ (0.001N) May 3, + FeCl ₃ (0.00002N) None None None
	C. May 8 (aqueous)	None	May 14, untreated May 14, + NaNO ₃ (0.001N) May 17, + NaNO ₃ (0.001N) May 19, + NaNO ₃ (0.001N) May 14, + FeCl ₃ (0.00002N) May 17, + FeCl ₃ (0.00002N) May 19, + FeCl ₃ (0.00002N) None None None None None None
P. caud. 6aII, March 28, 1919			

P. caud. 6aII, March 28, 1919	D. May 23 (aqueous)	None	May 26, + NaNO ₃ (0.001N) May 28, + NaNO ₃ (0.001N) May 26, + FeCl ₃ (0.00002N) May 28, + FeCl ₃ (0.00002N)	None None None None
	E1. June 6 (aqueous)	None	June 14, + 'tap-water' June 14, + NaNO ₃ (0.001N) June 14, + FeCl ₃ (0.00002N)	None None None
	E2. June 6, 'Na + Ca sol.' (0.01N) ¹	None	June 14, + FeCl ₃ (0.00005N) June 14, + FeCl ₃ (0.00003N) June 14, + 'Na + Ca' (0.01N) June 14, + 'Na + Ca' (0.02N) June 18, + 'Na + Ca' (0.01N) June 18, + FeCl ₃ (0.00002N)	None None None None None None
	A. April 11 (aqueous)	April 12: one pair of caudatum	April 14, untreated	None
	B. April 23 (aqueous)	None	April 25, untreated	None
	C. May 9 (aqueous)	'P. caudatum: May 14, 2-5% P. aurelia: May 13, casual May 14, casual May 16, 10-20% May 17, 5-10%	May 14, + NaNO ₃ (0.001N) P. caudatum P. aurelia May 14, + FeCl ₃ (0.00002N) P. caudatum, P. aurelia	20-30% 5% 30-50% Casual

¹ The 'Na + Ca solution' used in many experiments was composed of 50 parts N sol. of NaNO₃ + 5 parts N sol. of Ca(NO₃)₂ + 2 parts N sol. of NaHCO₃; the dilutions given being expressed in terms of the concentration of the NaNO₃.

TABLE 2

PARAMECIUM STRAIN	DORMANT CULTURE	DATE RENEWED; CULTURE MEDIUM	CONJUGATION IN THE RENEWED CULTURE
<i>P. caudatum</i> , <i>17aIII</i>	March 15, 1919	March 31 (aqueous)	April 10, about 30% April 11, about 20%
<i>P. caudatum</i> , <i>17aIII</i> (<i>P. aurelia</i> , <i>23i</i> added on March 31)	March 15, 1919	March 31 (aqueous)	<i>P. caudatum</i> : April 10, about 20% April 11, about 30% <i>P. aurelia</i> : April 12, one pair
<i>P. caudatum</i> , <i>17aIII</i> (<i>P. aurelia</i> , <i>13a</i> added on March 31)	March 15, 1919	March 31 (aqueous)	<i>P. caudatum</i> : April 10, casual <i>P. aurelia</i> : None

TABLE 3

(Each of the seven cultures of *Paramecium caudatum* listed in the first column was treated as here indicated)

<i>38b</i>	Dormant since	Conjugation in	Experiments with each race
<i>39a</i>	November 14,	culture <i>2a</i> , <i>44a</i>	as follows:
<i>44a</i>	1919	and <i>57a</i> (p. 351	March 9, + $\text{AlCl}_3(0.00003\text{N})$
<i>57a</i>		of paper)	March 9, + $\text{FeCl}_3(0.00002\text{N})$
<i>43a</i>			March 12, + $\text{AlCl}_3(0.00003\text{N})$
<i>2a</i>	Renewed March 3,		March 12, + $\text{AlCl}_3(0.00009\text{N})$
<i>8a</i>	1920		March 12, + $\text{NaNO}_3(0.001\text{N})$
			(Results essentially negative; p. 351 of paper.)

TABLE 4

DORMANT CULTURE	DATE RENEWED; CULTURE MEDIUM	CONJUGATION IN RENEWED CULTURE	SUBCULTURES 24 HOURS AFTER TREATMENT: CONJUGATION
<i>P. aurel.</i> , 13a II, March 28, 1919	A. April 11 (aqueous)	None	April 14, untreated None
	B. April 23 (aqueous)	None	April 25, untreated None May 3, + NaNO ₃ (0.001N) 5% May 4, untreated Casual May 10, untreated None May 10, + NaNO ₃ (0.001N) None May 10, + FeCl ₃ (0.00002N) None
	C. May 8 (aqueous)	May 13, casual May 14, 3-5% May 16, 10%	May 14, + NaNO ₃ (0.001N) None May 14, + FeCl ₃ (0.00002N) None
	D. May 23 (aqueous)	May 30, casual	May 27, + NaNO ₃ (0.001N) Casual May 27, + FeCl ₃ (0.00002N) None
	E1. June 6 (aqueous)	None	June 10, untreated None June 10, + NaNO ₃ (0.001N) None June 10, + FeCl ₃ (0.00001N) None
	E2. June 6, 'Na + Ca sol.' (= NaNO ₃ 0.01N)	June 9, casual	June 10, untreated Casual June 10, + 'Na + Ca' (0.01N) Casual June 10, 10 cc. culture + 1 cc. FeCl ₃ (0.0001N) 5% June 10, 10 cc. culture + 2 cc. FeCl ₃ (0.0001N) 20%

TABLE 5

DORMANT CULTURE	DATE RENEWED; CULTURE MEDIUM	CONJUGATION IN RENEWED CULTURE	SUBCULTURE 24 HOURS AFTER TREATMENT: CONJUGATION
<i>P. aurel.</i> , 14aII, April 11, 1919	A. May 13 (aqueous)	None	May 17, + NaNO ₃ (0.001N) May 31, + NaNO ₃ (0.001N) None None
	B. May 26 (aqueous)	None	May 29, + NaNO ₃ (0.001N) May 31, + NaNO ₃ (0.001N) None None May 29, + FeCl ₃ (0.00002N) None May 31, + FeCl ₃ (0.00002N) None
	C. June 14, 'Na + Ca sol.' (0.01N)	June 19, casual June 23, casual	June 19, + 'Na + Ca' (0.01N) June 19, + 'Na + Ca' (0.03N) June 19, 2 cc. culture + 1 cc. FeCl ₃ (0.0002N) Casual Casual June 21, + 'Na + Ca' (0.01N) 5% June 21, + 'Na + Ca' (0.03N) Casual June 21, 1 cc. culture + 1 cc. FeCl ₃ 3% (0.0002N) 3%
	DI. June 28 (aqueous)	None	
	D2. June 28, 'Na + Ca sol.' (0.01N)	July 3, 1-3% July 6, casual	July 6, + 'Na + Ca' (0.01N) July 6, + 'Na + Ca' (0.015N) None Casual

TABLE 6

PARAMECIUM STRAIN	DORMANT CULTURE	DATE RENEWED; CULTURE MEDIUM	CONJUGATION IN THE RENEWED CULTURE
<i>P. aurel.</i> , <i>11j</i>	July 24, 1919	A. September 19 (aqueous)	None
		B. September 19 NaNO ₃ (0.002N)	September 26, casual
<i>P. aurel.</i> , <i>11i</i>	July 24, 1919	A. September 25 (aqueous)	None
		B. September 25, NaNO ₃ (0.001N)	None
		C. September 25, NaNO ₃ (0.002N)	September 30, one pair
		D. September 25, NaNO ₃ (0.003N)	September 30, 3 to 5 pairs

TABLE 7

PARAMECIUM STRAIN	DORMANT CULTURE	DATE RENEWED; CULTURE MEDIUM	CONJUGATION IN THE RENEWED CULTURE
<i>P. aurel.</i> , <i>1bI</i> (stock culture)	July 24, 1919	A. September 30 (aqueous)	October 2, 5-10%
		B. September 30, NaNO ₃ (0.0025N)	October 2, casual October 3, 5%
		C. September 30, NaNO ₃ (0.005N)	October 2, casual October 3, 1-3%
		D. September 30, 'Na + Ca' (0.005N)	October 2, casual October 3, casual
<i>P. aurel.</i> , <i>3d</i>	July 24, 1919	A. October 4 (aqueous)	None
		B. October 4, NaNO ₃ (0.001N)	October 8, 1-3% October 10, 1-5% October 12, 10-15%
		C. October 4, NaNO ₃ (0.002N)	October 8, 5-10% October 13, 1-3%

TABLE 8

Showing the number of divisions per line for five-day (and ten-day) periods in several lines of *Paramecium aurelia* (11g and 1b) and *P. caudatum* (38a), growing in various culture media (see explanation of tables). The organisms were cultivated on depression slides, renewed daily with fresh culture medium of hay infusion in distilled water, with or without the salts mentioned

PARAMECIUM STRAIN; DURATION OF EXPERIMENT		AQUEOUS MEDIUM						Na + Ca MED. (0.005N)						NaNO ₃ MED. (0 005N)					
		lines						lines						lines					
		1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
11g	1st. 5 days 2d. 5 days	9	9	9	9	9		13	13	13	13	13							
		7	7	8	10	9			9	11	9								
	10 days	16	16	17	19	18		22	24	22									
38a	1st. 5 days 2d. 5 days	6	7					11	10	10	11								
		6	4					9	10	10	9								
	10 days	12	11					20	20	20	20								
1b	5 days	4	7	5	7	7	5	10	12	11	11	12	11	11	13	13	10	10	13

VITA

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